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This is a summary of the program of work beginning in 1983 and addressing the issue of reversible to non-reversible transitions in brain relevant allosteric proteins studied and modeled as far-from-equilibrium dynamical systems. Stimulated by the issues not involving co-valent bonding in the aging phenomenon of cholinergic neurotoxins, a polypeptide-psychotropic drug strategy for "buying time" vis-a-vis manomolecular stability is suggested. A new, low dimensional ligand-receptor coding scheme was developed in collaboration with Roger Guillimin. New work on a simular scheme involving a channel protein design is now on-going in collaboration with Maurice Montal.

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MACROMOLECULAR INSTABILITIES AND DYNAMICAL CODING IN BRAIN ENZYMES, POLYPEPTIDE LIGANDS, POLYPEPTIDE AND CHOLINERGIC RECEPTORS, AND SODIUM AND CHOLINERGIC CHANNEL PROTEINS-A FINAL REPORT

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Brief Review of the Context

We were stimulated to pursue our work on "far-from-equilibrium" kinetic studies on brain enzymes, tryptophan and tyrosine hydroxylase, involving reversible and irreversable transitions in kinetic activity states in the context of the cholinergic toxin problem with its multiple state transitions; the suggestion that co-valent bonding was only part of the story in the "aging" process. Little fundemental work on neural system enzymes with respect to nonlinear behavior was available so we felt, though our work *at that time* was not directly related to the to the cholinergic neurotransmitter family, the phenomena and its theory might offer other than (almost equally toxic) competitive inhibitors as the only alternative in a biological neurotoxic defense strategy. In a very general way, we wanted to learn how to "play for time" with brain-related allosteric proteins so that more conventional blocking agents could be given in lower doses over longer times in protective and/or inhibiting ways.

We had discovered that tetrahydrobiopterin, the common cofactor in brain biogenic amine synthesis, was very far from saturating making it possible to study kinetic instabilities and large fluctuations in vitro with possible in vivo relevance.

Following several studies under base line conditions, we began to examine the interaction with polypeptide and ion ligands. Was it possible, for example, to "talk to proteins" with ligands which could dynamically (not structurally) protect neural-relevant proteins long enough for other, less toxic chelating agents (for example) to protect against the chemical assault.

Dynamical systems research had evolved at an accelerating rate with both a new theory of motions (chaos not noise) and reliable measures of this behavior (such as Hausdorf measure, entropies,

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characteristic exponents, power law spectra, symbolic dynamics, high moments, and qualitative phase portraits) such that this technology might be brought to bear on this problem. We have developed an extensive soft-ware system for both simulation of these processes on analog and digital computers using dynamical equations (including those of the reduced Hodgkin-Huxley variety) as well as data-analytic tools which have been and are being applied to such problems as "dynamical versus structural death" in biological systems. For example, strict periodicity in the time variational behavior of an enzyme, a receptor, a neuron, the heart, the EEG, and even hydrostatic pressure in the kidney is a sign of loss of regulatory potential. This is our model for the "desensitization" that precedes failure in a system. The Shannon (second) theorem says that the receiver must have higher entropy than the sender for reliable communication to occur. Bifurcation from chaos to order may be the critical dynamics in irreversability.

A second and related problem ("how can we talk to proteins" with ligands in such a way to alter dynamical scenerios, even those induced by eventually covalently bound ligands) is the language of ligand-membrane receptor communication. This issue is now made more difficult by evidence that membrane protein dynamics are chaotic ("1/f noise" across many time scales and not the Lorentzians of single channel life times) and ligand binding curves that manifest "multiple saturation plateaus" (if reaction conditions are explored over a sufficient range). Altogether these findings make the structural "lock-and-key", single discrete perturbation causing simple single channel open and close processes imagery less attractive. The fundemental problem, reliable communication via chaotic dynamics has been the cutting edge of the ergodic theory of dynamical systems over the past ten years--not without applications since IBM and XEROX use "chaotic" packing of information into amorphous solids in their magnetic tape mediated computer and communication machinery.

We have used the remarkable findings that the dynamics of nonlinear systems in low dimensional hyperspace produce only a finite number of transitions (bifurcations) and that "symbolic dynamics", coding the trajectories via the sequence of phase spaces occupied by so-called chaotic dynamics, has produced a rigid family of sequences (called kneading sequences or U(universal)-sequences) that can be used to code polypeptide chains and a wide family of proteins including enzymes, receptors, and most recently, those of transmembrane ion channels. As will be seen below, we have made successful predic-

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tions of ligand-receptor interactions using this approach in collaboration with the laboratory of Roger Guillimin and are now beginning to work on transmembrane and channel protein sequences with Maurice Montal. We feel a not insignificant advance has been made in the direction of another way to think about brain-related proteins dynamics and how communication between polypeptides and proteins may involve coding processes on a low dimensional manifold; perhaps only one dimension. More generally, we see a rather large broadening of the meaning of "allosteric", here suggesting phase transitions in global dynamics induced by an aggregate of influences rather than specific distant regulatory sites.

Brief Summary of the Work Represented by Twelve Enclosed Papers Supported in the Contract

1. From intermittency to transitivity in neuropsychobiological flows; Mandell, A.J.; Am. J. Physiol. 14:R484-R494, 1983.

The now classical "strange attractors" of the Lorenz and Rossler differential equations and a family of one-parameter maps of the real line are used to model two types of common neurobiological processes: random discharges and bursting. Examples of the new kinds of measures noted above are explained and a number of (roughly) comparable phenomena found in neurophysiological and neurochemical systems are portrayed. This "sets up the game" for the future work.

2. From chemical homology to topological temperature: a notion relating the structure and function of brain polypeptides; Mandell, A.J. Synergetics of Brain; Springer-Verlag. Ed. Haken et. al.; 1983)

The idea is developed that stability not energy governs information transport in intrinsically unstable brain molecules. Ligands restrain autonomous motions and induce phase transitions. Ligand-protein recognition involves "resonance" between similar hydrophobic amino acid "loops" which curl around at regions of hydrophobic minima. Certain polypeptide families are examined in this context and the actions of a group of equally potent corticotrophic releasing factors that differ by half in amino acid specificity seem to be explained by this approach.

3. TRH influences the pterin cofactor- and time-dependent instabilities of rat raphe tryptophan hydroxylase activity assessed under far-from- equilibrium conditions; Knapp, S. and Mandell, A.J. Neurochem. Internat. 6: 801-812, 1984.

This series of experiments demonstrates the action of a tri-peptide, thyrotropic releasing hormone, on the far-from-equilibrium kinetics of rat raphe tryptophan hydroxylase as model of how such ligand-protein interactions change the patterns of time-dependent instabilities rather than amount of product produced per unit time. Our somewhat controversial position, that there are no rates in the form of linear differential equations of the classical kinetic type, but rather global nonlinear dynamical patterns of behavior modeled by maps and flows on low dimensional manifolds is seen here in the form of power law spectra, autocorrelation graphs, and Hausdorf dimensions on real data. The peptide induced changes seen previously using ion ligands (lithium versus calcium) and cholinergic antidepressant drugs. Transitions from a high entropy, "white noise" pattern to that of slow coherence addresses the issue outlined above concerning irreversibility. A control group of studies indicates these effects are unrelated to facilitation or inhibition of thermally induced denaturation.

4. Non-equilibrium behavior of some brain enzyme and receptor systems; Mandell, A.J. Ann. Rev. Pharmacol. Toxicol. 24: 237-274,1984.

Evidence is summarized for role of dynamical instabilities and hydrophobic coding in a variety of contexts involving brain proteins. Particular attention is paid to the regulatory literature involving brain tryptophan and tyrosine hydroxylases (as model allosteric brain proteins) and a few moderately well charactertized ligand binding preparations. Again, the theme, dynamics of rate and not rate constitute the important aspects of instability, transitions, and coding.

5. Relationships between drug-induced changes in tetrahydrobiopterin and biogenic amine concentrations in rat brain. J. Pharmacol. Exper. Therap.(JPET); Lee, E.H.Y. and Mandell, A.J.; 234:141-146, 1985.

This paper is principally of a technical nature; a re-examination of our earlier findings using a double-enzyme assay, now upgraded to a high-performance liquid chromatographic technique, to justify our far-from-equilibrium reaction conditions used to study and model our biogenic amine biosynthetic brain enzymes. In fact, tetrahydrobiopterin is in remarkably low concentrations in biogenic amine systems with respect to the conventional in vitro kinetic studies which produce equilibrium and saturation kinetics. The stability of this measure across drugs and the unique amphetamine effect is confirmed

using the more specific and precise technique. If neural processes have coding as their primary function, the entropy required for such a task would appriori demand nonequilibrium dynamics.

6. The hyperbolic helix hypothesis: S.. ..eton's fractal measure on the hydrophobic free energy mode distributions of allosteric proteins. Mandell, A. J. in Fractals in Physics (ed. Pietronero and Tosatti); 1986.

This constitutes the beginning of the development of a nonlinear model for the allosteric protein (using the Cartwright-Littlewood-Levinson- van der Pol highly nonlinear, forced dissapative oscillator--not unrelated to some reduced forms of the H and H equations). Hydrophobic free energy is introduced as "surface tension" using the work of Tanford on lipid-aqueous partitions and cavity size of amino acids as its basis. Percent helix using optical methods, Stapleton's "fractal" measure of x-ray maps and statistical studies of hydrophobic sequences on some proteins are used as the data base. Some thermodynamic data is considered. I consider this effort quite primitive but it contains the roots of the later work.

7. Prime times: the distribution of singularities in hydrophobic free energy of proteins. In Perspectives in Nonlinear Dynamics; (ed. Shlesinger et al). World Scientific. 1986.

This represents a year's work in which I try to continue the theme of above more extensively with the proposition being that the behavior of allosteric proteins can be represented as as chaotic dynamics on low dimensional manifolds and that given this view of structure and function in ligands and proteins, we can derive a finite set of coding sequences generated by specifiable transformation groups. Universality (structural stability) of dynamical systems in low codimension is exploited in an effort to argue that many little mistakes can be made in this form of communication involving inevitable singularities without loss of reliability or capacity for innovation. We are still working on this theme and will use some new computer models and examples in a paper we are finishing now to try clarify these still murky, and incompletely developed issues. The implications of this work (if of value) for molecular biological coding is clear.

8. Sonication, calcium, and peptides have systematic effects on the nonlinear dynamics of tyrosine hydroxylase activity. Russo, P. V. and Mandell, A. J. Neurochem. Int. 9: 171-176. 1986.

This is a *very carefully executed* companion paper to the TRH-tryptophan hydroxylase study (#3) discussed above demonstrating for caudate dopaminergic tyrosine hydroxylase studied under conditions of far-from-equilibrium tetrahydrobiopterin concentrations that *specific* peptides and ion-ligands can both confer and destroy coherence in the kinetic fluctuations using power spectra and Hausdorf measure. Example of the use of non-parametric statistics on "momentless" data distributions using these descriptors serves to exemplify approaches to data which most laboratories (including us in the past) might have thrown away. It again deals specifically with the issue of complexity and coherence in dynamics with respect to chemical communication.

9. Polypeptide hormones and receptors: participants in and products of a two parameter, dissipative, measure preserving, smooth dynamical system in hydrophobic mass energy. Mandell, A.J. in Chaos in Biological Systems (ed. Degn et al); Plenum. 1987.

The dynamical equation for the global dynamics of polypeptides and proteins motions introduced in (#6), developed to some extent in (#7) is now used to construct phase space portraits of protein dynamics and their power spectra which show how parameter-sensitive changes can systematically generate most of the known polypeptide broad-band modes. Examples are given of mode-mode isomorphisms between polypeptide ligands and their receptors. A prediction of the action of the newly characterized fibroblastic growth factor family (quasi-isomorphic with ribonuclease and therefore a potential regulator of it) was confirmed in that it was found to stabilize template. Two papers (Zeyton et al. Endocrinology; March, 1988) gives an account of these studies and the success of these predictions

10. Geometric universality in brain allosteric protein dynamics: complex hydrophobic transformation predicts mutual recognition by polypeptides and proteins. Ann. N.Y. Acad. Sci. 504: 88-117, 1987.

This recasts the problem of near-phase transition status of allosteric proteins and their apparent scaling behavior into another phase space: that of non-Euclidian geometry. There is ample evidence that many systems are conformally invariant when critical and the bounds of such spaces (if extended) are non-Euclidian. Coding on hyperbolic manifolds (Thurston's geodesic laminations) is another form of universality. Complex transformation of the dynamics of differential systems at the border of topological stability, the breakdown of the torus for example, has been approached by many workers beginning

with Poincare. The differential equation for these dynamics is studied further with a portrait of mode locking zones, Arnol'd tongues, is shown and a some polypeptide data is studied. Most notably the AIDS virus coat which was statistically dominated an "8 mode" even when the specific amino acids were almost entirely different.

11. The source and characteristics of normal modes in molecular biology. Mandell, A.J. In Chaos and Order in the Natural Sciences; (ed. M. Velarde). Springer- Verlag. Due out in 1988.

After "cleaning up" the previous thermodynamic development a bit, I use the "U-sequence"--- found by Metropolis, Stein, and Stein in all single maximum, nonlinear maps of the real line, to generate polypeptides that resemble those in the real world. This moves from a co-dimension two differential equation used in (#9) to a one-dimensional map like a print-sequence down a polysome tunnel such that the next addition is a function of the previous one and the place in parameter space of the global dynamics. The implication here is that there may be a global "field" initial approximation in the nucleotide-amino acid information transport process. I devised a simple graphics representation which seemed to portray these kinds of differences between different peptides and built intermittent decay (instability) into the mode generating equation parameters so that the intermittency of dominant hydrophobic modes seen in real sequences could be found. The full family of "mode four" polypeptides with very similar actions are summarized to continue to add to our list of examples of the value of power spectra of hydrophobic broad band modes in the characterization of structure and function in polypeptides and receptors.

12. An unstable singularity theory of molecular biological coding; calcitonin's structures and potencies. In Dynamic Patterns in Complex Systems (ed. S. Kelso et al); World Scientific Press; 1988. Out in April.

This suggests the use of a Pareto-Levy distribution and its convolutional stability as a statistical representation of the unstable singularity distribution characteristically found in polypeptide chains. This becomes important in a revision of Shannon's definition of information since it requires finite moments (that information deviates from) and that there are not finite moments in macromolecular dynamics or coding sequences. A more modern approach is taken to water as "picosecond jello" and the

peptide generating algorithm is turned to the problem of designing a calcitonin molecule. The large naturally occuring calcitonin family is examined with perhaps the nicest isomorphisms among power spectra of family members found yet as a result. Fractal measures are being used on the sequences suggesting that higher hydrophobicity goes with higher Hausdorf dimension such that curling up too much (on itself) reduces the external mode availability and weakens the dose-response potency of the analog. That is our theory as to why the salmon is a stronger calcitonin than man in man.

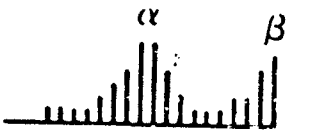
Current Work Involving Cholinergic Receptor and Sodium and Cholinergic Transmembrane Receptor Proteins

We have just begun a collaboration with Maurice Montal of our Department of Physics involving the custom design of channel proteins based on our hydrophobic sequence analytic methods. It is much too early to make any definitive statements however I have appended two sets of figures indicating some interesting preliminary findings.

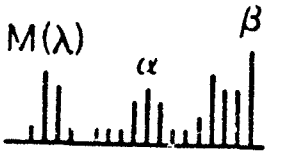
1. We see that the power spectra of the alpha monomer of three cholinergic receptors dominated alpha helices (3.3-3.6 residue modes) and beta strands (2.0-2.4 residue modes). Of interest is that the snake neurotoxins that bind and inactivate cholinergic receptors also have these dominant modes. This may be another interesting example of ligand-receptor broad band isomorphism similar to that found in some polypeptide receptors noted above.

2. Preliminary analysis of sodium channel protein polypeptide segments from four sources demonstrate a similar (and a bit unusual) transmembrane mode that is "longer" than beta strands and "shorter" than the usual polypeptide alpha helix. Very high average hydrophobicity (1.70 kcal/mol/res) is characteristic of those peptide segments seen in transmembrane parts of proteins like the EGF receptor and the high d_H, Hausdorf dimension of the sequence, is characteristic of chains with higher hydrophobicity. It is Montal's theory that there may be a fundamental building block for channel proteins (proposed also by Weber and Salemme) involving such a structure. We are in the process of comparing a wide range of trans and intra-membrane proteins to see if this pattern is unique. Design of artificial ones (already done with success by Montal) will continue. I will use my computer design algorithm on this task as well.

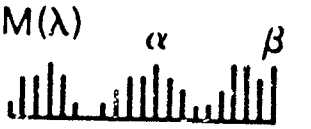
(a) Electrophorus



(b) Torpedo



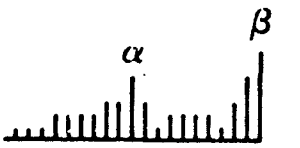
(c) Calf 49-K



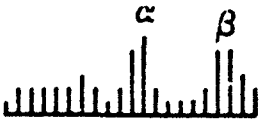
Neurotoxins

Cholinergic Receptor

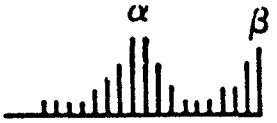
α-Cobratoxin



Erabutoxin



Electrophorus



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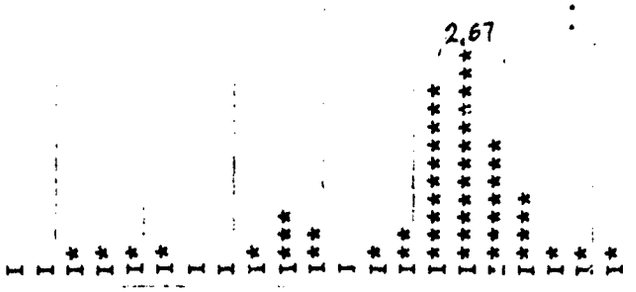

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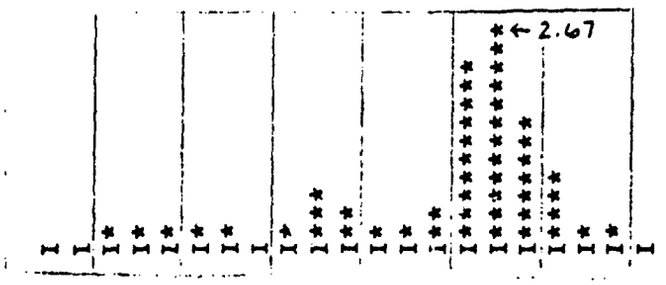

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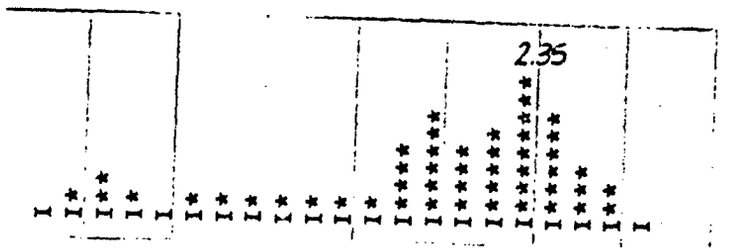
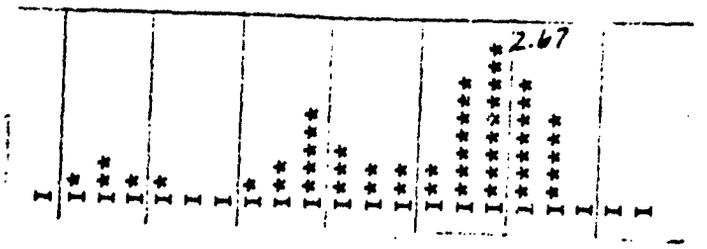
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